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Histological features of interrenal and chromaffin cells in relation to seasonal testicular activities in *Notopterus notopterus* (Pallas, 1769)

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Abstract: The adrenal gland of *Notopterus notopterus* (Pallas) consisted of interrenal and chromaffin cells, distributed around the main branches of posterior cardinal vein in most anterior part of the pronephric kidney. Seasonal histological characteristics of the interrenal and chromaffin cells in relation to testicular activity of *N. notopterus* were studied by adopting different staining techniques. The cytophysiological features of the interrenal, chromaffin cells and various spermatogonial cells were observed during different reproductive phases. In the present investigation, the relationships between the changes in the interrenal and chromaffin cells along with the spermatogenic cells at different periods of the breeding cycle were discussed.

Keywords: Cyclical changes, Adrenal, Testis, *Notopterus notopterus*.

Introduction

In has been intimated that the interrenal cells are homologous to the mammalian adrenal cortex, whereas the chromaffin cells are homologous to the mammalian medullary cells (Gazola et al. 1995). In teleosts, adrenal glands are made up of two tissues: interrenal and chromaffin, intermingling to varying degrees depending on the species, they take up different positions and associated with the posterior cardinal vein and its tributaries within the head kidney (Nandi 1965; Hanke & Kloas, 1995; Rocha et al. 2001; Sampour 2008). Aminergic chromaffin and interrenal steroidogenic cells can be blended neighboring on entirely dissevered lining of the endothelium of the venous blood vessels or located in its close proximity (Gallo & Civinini 2003). Seasonal possible cellular changes of interrenal and chromaffin cells in relation to reproductive activities have been studied in several teleosts (Civinini et al. 2001; Rocha et al. 2001; Gallo et al. 2004; Chakrabarti & Ghosh 2014).

As the interrenal cells and gonads are the essential elements involve in steroidogenesis along with other physiological processes, the aim of the present work is to examine more precisely the correlative changes between the activities of the interrenal and chromaffin cells with testis during different reproductive phases in *Notopterus notopterus* (Pallas) by adopting histological techniques. *Notopterus notopterus* is a carnivorous freshwater teleost and this fish is also considered as palatable and economically important fish species. Therefore, it would be interesting to study in details the different functional status of interrenal and chromaffin tissues in correlation with the testicular development.

Materials and methods

Adult live male specimens of *N. notopterus* (length 21-23cm and weight 150-200g) were procured from local freshwater body of particular area during the second week of every month from January to December 2012. The fishes were pretreated with 1% methylene blue for 10 min. for disinfection and then released into a well aerated aquarium (120.90x60.92x60.92 cm) having dissolved oxygen

4.5-5.8mg/L, pH 6.8-7.2, temperature 25-31°C in summer and 18-22°C in winter. The fish were acclimatized for 5 days by feeding finely chopped goat liver and Tubifex. Data on total body weight and testis weight of ten fish were taken to calculate the mean gonadosomatic index (GSI) using the following formula:

$$GSI = \frac{weight\ of\ the\ testes}{weight\ of\ the\ fish} \times 100$$

For histological studies after decapitation of the fish, the head kidneys and the testes were quickly removed, cut into small pieces and were fixed in aqueous Bouin's fluid for 18h. Subsequent to dehydration the tissues were embedded in paraffin wax of 56-58°C under a thermostat vacuum paraffin-embedding bath for 1h. All the tissues were serially sectioned at 4µm and stained with Mallory's triple, Delafield's haematoxylin-eosin and Iron-alum haematoxylin stain. From the histological preparations the measurement of interrenal and chromaffin cells, diameter of various spermatogenic cells were measured with the help of reticulo micrometer and ocular micrometer, respectively.

Results

Histologically, the adrenal gland of *N. notopterus* consists of interrenal and chromaffin cells which are separated from each other and distributed around the main branches of posterior cardinal vein in the anterior kidney. The interrenal and chromaffin cells are surrounded by plasma membrane, which separates the cell from adjacent cells (Figs. 1, 3, 4, 7). The cytoplasm of the interrenal cells shows a basophilic colouration with Mallory's triple stain and haematoxylin-eosin stain. The chromaffin cells are eosinophobic and round in shape. The interrenal and chromaffin cells are characterized with centrally placed spherical conspicuous nuclei.

The testis of *N. notopterus* is single, oval in shape and solid in manifestation. The testis is typically made up of an anastomosing network of different shaped lobules (Figs. 2, 8, 11) surrounded by lobule boundary wall. The diameter of the lobule also varies greatly during the different periods of the season. Interstitial cells in association with blood cells are visible in the interlobular spaces (Figs. 5, 6, 8). Five types of germ cells viz, spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa are identified from the periphery towards the lumen of seminiferous tubules in various reproductive phases.

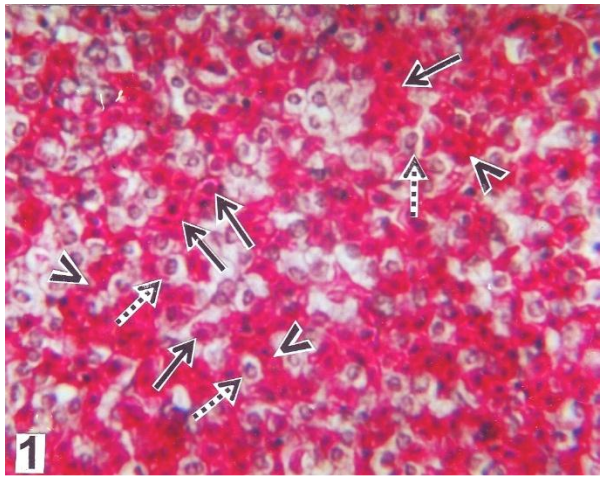

Fig.1. Photomicrograph of section of adrenocortical tissue in *N. notopterus* showing interrenal (IR) (solid arrows) and chromaffin cells (CH) (broken arrows) associated with blood sinusoids (arrow heads) during growth phase. Note acidophilic cytoplasm of interrenal cells (Delafield's haematoxylin-Eosin: H&E) X400.

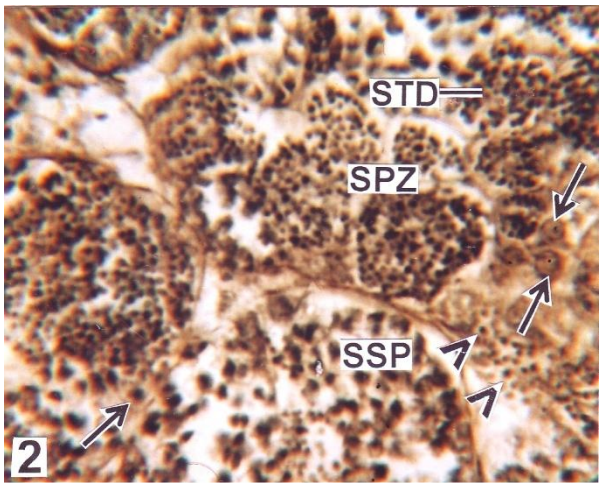

Fig.2. Photomicrograph of section of testis in *N. notopterus* showing spermatogonia (arrows), primary spermatocytes (arrow heads), secondary spermatocytes (SSP), spermatid (STD) and spermatozoa (SPZ) during growth phase (Iron alum haematoxylin: IA) X400.

(1) Spermatogonia: These are the largest of all the

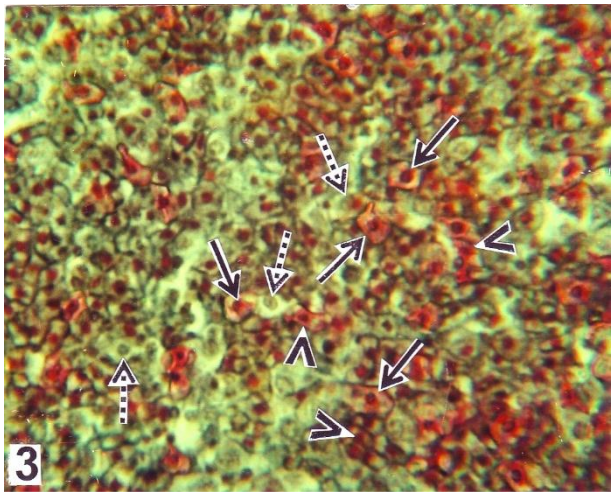

Fig.3. Photomicrograph of section of adrenocortical tissue in *N. notopterus* showing increment of the size of IR (solid arrows) in between CH (broken arrows) during maturation phase. Note blood sinusoids (arrow heads) in between IR and CH (Mallory's triple: MT) X400.

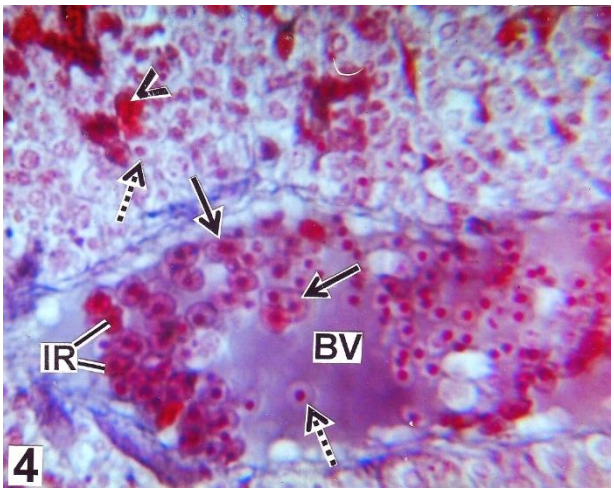

Fig.4. Photomicrograph of section of adrenocortical tissue in *N. notopterus*. Aggregation of enlarged IR (solid arrows) within the wall of blood vessel (BV) during maturation phase. Note few CH cells (broken arrows) within BV and outside the wall of BV. Arrow head indicates blood cells (H&E) X400.

spermatogenic cells and attached to the inner margin of the lobule boundary wall (Figs. 2, 5, 12). They are almost spherical and the diameters ranging from 10.89±0.14×9.0±0.09µm in outline having small amount of chromophobic cytoplasm and centrally placed nuclei (Figs. 2, 12). The spermatogonia undergo several mitotic divisions and give rise to a large number of primary spermatocytes.

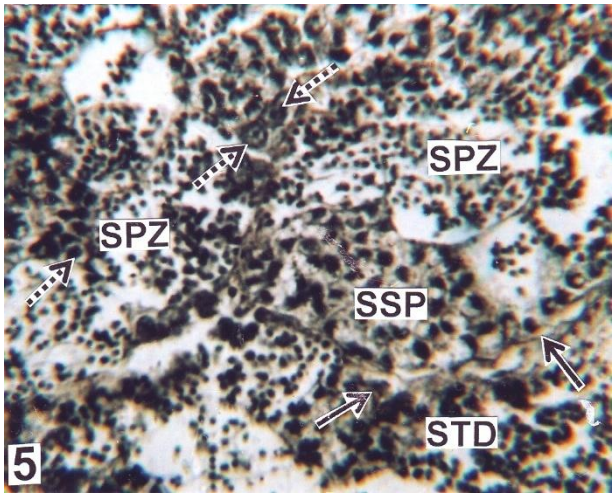

Fig.5. Photomicrograph of section of testis in *N. notopterus* showing proliferation of SSP, STD and SPZ during end of maturation phase. Note few SPG (broken arrows) and interstitial cells (solid arrows) (IA) X400.

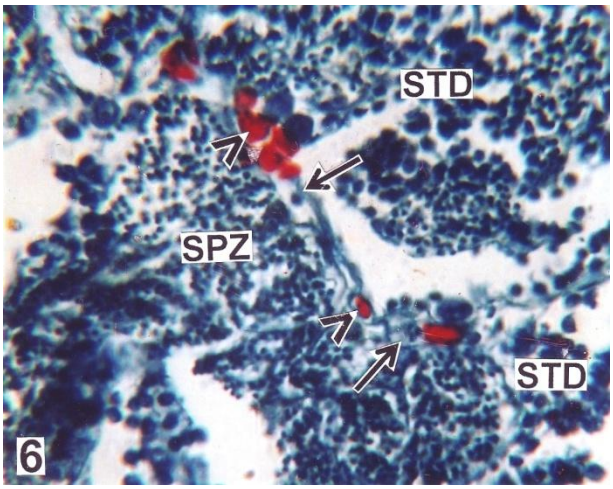

Fig.6. Photomicrograph of section of testis in *N. notopterus* showing cyst of STD and packed SPZ within the testicular lobules during spawning phase. Note active interstitial cells (solid arrows) associated with BV (arrow heads) (MT) X400.

(2) Primary spermatocyte: The primary spermatocytes contain relatively lesser amount of chromophobic cytoplasm and the nucleus is deeply stained with haematoxylin. They are spherical or oval in outline with diameters ranging from 8.32±0.06×6.4±0.13µm. (Fig. 2).

(3) Secondary spermatocyte: The secondary spermatocytes are smaller than the primary spermatocytes. The cytoplasm of the cells is difficult

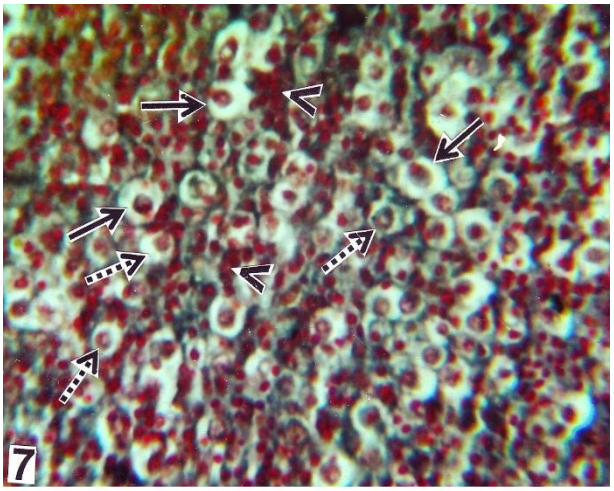

Fig.7. Photomicrograph of section of adrenocortical tissue in *N. notopterus* showing depleted cytoplasm and atrophied nucleus of IR (solid arrows) and CH cells (broken arrows) adjacent to sinusoid (arrow heads) during spawning phase (MT) X400.

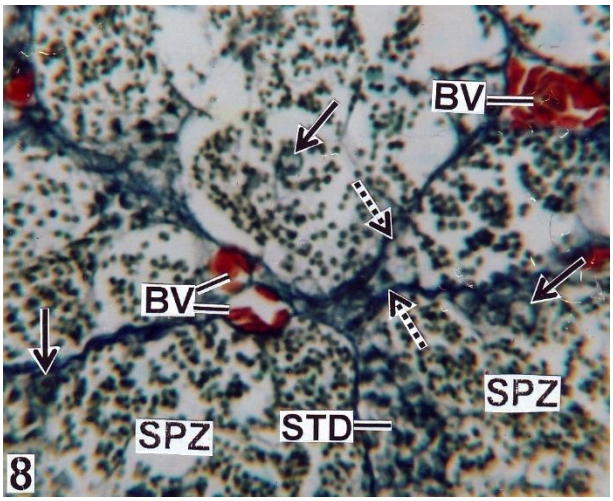

Fig.8. Photomicrograph of section of testis in *N. notopterus.* Anastomising testicular lobules packed with SPZ and cyst of spermatid (STD) during spawning phase. Note few SPG (solid arrows) within the lobule, BV and IC (broken arrows) in the interlobular spaces (MT) X400.

to distinguish. These are nearly spherical in shape with their diameters ranging from 4.8±0.08×3.2±0.05µm. The nuclei are much darker (Figs. 2, 5).

(4) Spermatids: The spermatids are further reduced in size and are stained deeply with haematoxylin. The nucleus is crescent shaped and approximately 2.61±0.10×2.20±0.05µm in size (Figs. 2, 6, 8).

(5) Spermatozoa: There are the eventual consequence

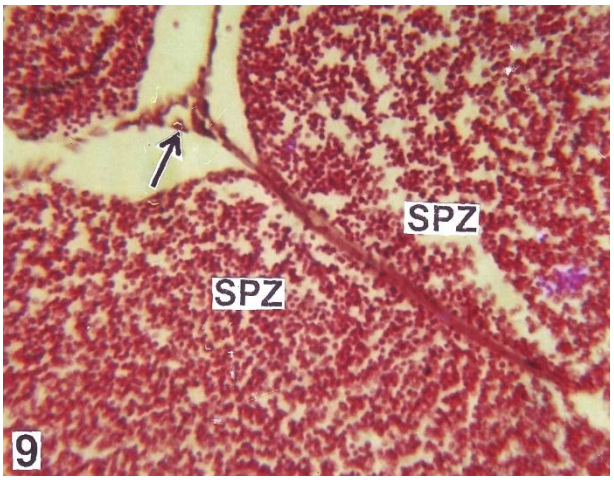

Fig.9. Photomicrograph of section of testis in *N. notopterus* showing packed SPZ within the lobules having thin boundary wall. Note IC (arrow) in the interlobular space (H&E) X400.

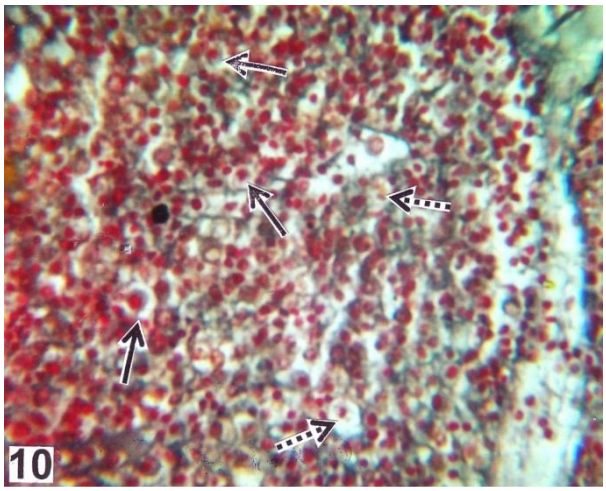

Fig.10. Photomicrograph of section of adrenocortical tissue in *N. notopterus* showing reduction in size of IR (solid arrows) and CH cells (broken arrows) during post-spawning phase (MT) X400.

of spermatogenesis and smallest one of all the spermatogenic cells with an average diameter of 1.28±0.04×1.18±0.06µm and has strong affinity to haematoxylin (Figs. 5, 6, 8, 9).

Interstitial cells: These cells are triangle or elongated in shape and associated with the blood vessels in the interlobular spaces. The interstitial cells undergo variations in shape and size during different reproductive phases (Figs. 5, 6, 8, 9).

Cyclical changes of interregnal and chromaffin cells in relation to spermatogenesis: The activities of the interrenal and chromaffin cells are found to undergo

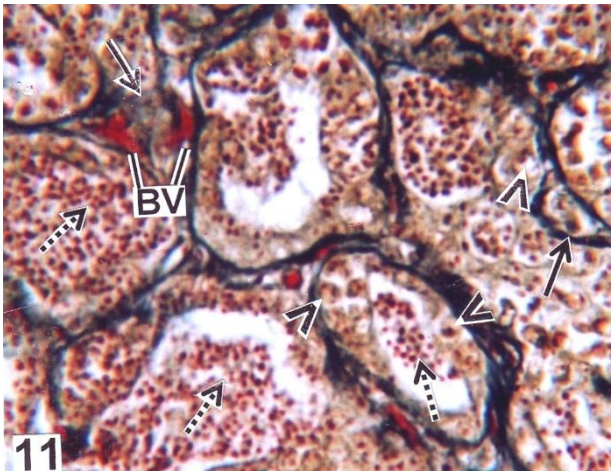

Fig.11. Photomicrograph of section of testis in *N. notopterus*. Anastomising testicular lobules having thick boundary wall during post-spawning phase. Note SPG (arrow heads) in the inner lining of lobules, residual SPZ (broken arrows) and IC (solid arrows) adjacent to BV (MT) X400.

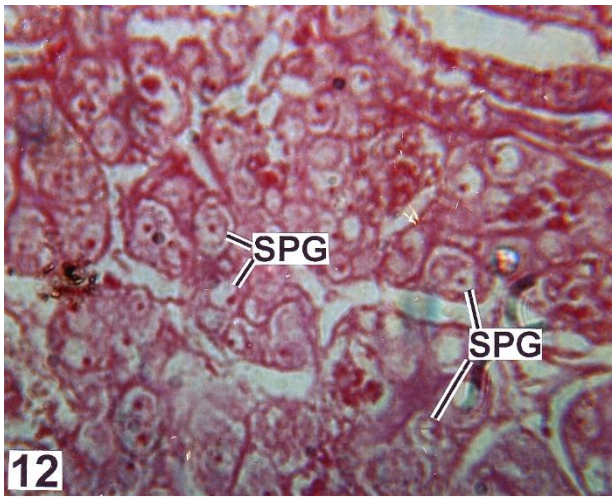

Fig.12. Photomicrograph of section of testis in *N. notopterus* showing predominant SPG in the testicular lobules during end of post-spawning phase. Note prominent nucleus and chromophobic cytoplasm in SPG (H&E) X400.

correlative seasonal changes along with the different reproductive phases. The changes in the activities of adrenocortical tissue have been studied by considering their number, distributional pattern and the cell size. However, on the basis of gonadosomatic index (GSI) and the occurrence of various spermatogenic cells, the reproductive phases of *N. notopterus* can be dissevered into four phases: growth (December to February), maturation (March to May), spawning (June to August) and post-spawning and resting phases (September to October).

Growth phase (December to February): During this phase in male, the interrenal cells are oval or polyhedral in shape arranged in clusters adjacent to the blood vessels and sinusoids. The cellular diameters of interrenal cells ranging from 2-4µm. The cytoplasm is acidophilic in nature when stained water haematoxylin-eosin. Chromaffin cells are oval or rounded in appearance having clear cytoplasm intermingled with the cortical cells and associated with blood vessels. The diameter ranging from 3-5µm (Fig. 1). In the present investigation in testis the value of gonadosomatic indices (GSI) ranges from 0.142±0.05 to 0.32±0.16. The spermatogonial cells appear to divide. At the end of this phase primary, secondary spermatocytes, spermatids are noticed. New spermatozoa are found in clusters (Fig. 2).

Maturation phase (March to May): During this phase the clusters of interrenal and chromaffin cells are oriented encircling the sinusoids. When stained with Mallory's triple the cytoplasm is lightly acidophilic, while the nucleus is basophilic and mostly rounded. The chromaffin cells are arranged in groups, in between interrenal cells and renal sinusoids (Fig. 3). The diameter of interrenal cells (6.80±0.18µm) and chromaffin cells (6.10±0.11µm) increased and undergo hypertrophy during this phase. The interrenal and few chromaffin cells are present in clusters along the wall of the blood vessels (Fig. 4). The testis when enters in maturation phase, the GSI aligns from 0.516±0.03 to 0.85±0.06. The testis is typically made up of an anastomosing network of different shaped lobules (Fig. 5). All types of spermatogenic cells appear in this phase. The spermatogonia decrease in number. The spermatocyte cells are transformed into spermatids and spermatozoa. The interstitial cells become active and the size of the cells varies from 8.5±0.12×9.8±0.10 µm (Figs. 5, 6). During the end of this phase distended seminiferous lobules totally packed up with cysts of spermatid and spermatozoa closely associated with blood vessels (Fig. 6).

Spawning phase (June to August): In the spawning phase the interrenal cells undergo momentous changes. Cortical cells are round or oval in shape with depleted cytoplasm. In the early spawning phase, the interrenal cells contain chromophobic cytoplasm provided with hypertrophic nuclei (Fig. 7). Diameter of interrenal cells during spawning season varies from 7.15±0.08 to 7.75±0.11 µm. Chromaffin cells are comparatively round or oval in shape with centrally placed large nucleus and chromophobic cytoplasm. Diameter of chromaffin cells varies from 6.17±0.09 to 6.85±0.18µm. In the testis the GSI value is recorded to 1.58±0.01 in June 1.33±0.16 in July and 1.16±0.02 in August respectively. The testicular lobules attain a maximum width at this stage and are packed with spermatids and spermatozoa. Interstitial cells are found to be associated with the blood vessels (Fig. 8). In the late spawning phase the thickness of the testicular wall decreases considerably and the lobules are packed with spermatozoa (Fig. 9).

Post-spawning and Resting phase (September to November): The interrenal and chromaffin cells are attenuated in size and the cytoplasm becomes vacuolated. The size of the interrenal cells varies from 5.60±0.02 to 5.70±0.07 µm having hypertrophic nuclei and associated with blood sinusoids. The cytoplasm of chromaffin cells assumes a vacuolated nature due to the degranulation. The diameter of chromaffin cells varies from 4.20±0.14 to 4.85±0.08µm (Fig. 10). In the testis the GSI value ranges from 0.16±0.01 to 0.12±0.04. The diameter of the tubules decreases and the boundary wall gradually becomes thicker. Residual spermatozoa are dispersely present along with spermatogonial cells. The inconspicuous interstitial cells are present in between the tubules (Fig. 11). During resting phase the spermatogonia are the predominant germ cells and are compactly arranged within the lobules (Fig. 12).

Discussion

The interrenal cells of teleosts are homologous to the mammalian adrenal cortex and are well established as the source of adrenocortical steroids (Chester-Jones & Philips 1986). In the present investigation, the interrenal and chromaffin cells in *N. notopterus* are located within the hand kidneys and are mainly associated with the posterior cardinal veins and their tributaries. Similar observations were also made by Civinini et al. (2001), Sampour (2008) and Abdel-Aziz et al. (2010) and they considered to be typically teleostean. In *N. notopterus* the interrenal cells are comparatively larger than chromaffin cells. These are basophilic in nature. The chromaffin cells contain pale cytoplasm and slightly basophilic nuclei.

Though the physiological role of adrenocortical tissue during the period of sexual maturation and spawning is not clearly understood but the significant hyper and hypo activity of interrenal tissues corresponds with the breeding and non-breeding phases of *N. notopterus*. In the present study, the interrenal and chromaffin cells exhibited changes concomitant with the testicular activities in *N. notopterus*. The accumulation of cytoplasmic contents of interrenal and chromaffin cells coincided with the transformation of various germ cells and beginning of spermiation. The spermatogenetic activities in testis continued up to the spawning period. During this period hypertrophied and vacuolated interrenal cells were encountered which possibly released their content for final maturation of the spermatocytes. The results obtained from the present investigation in *N. notopterus* chromaffin cells were more or less unaltered in appearance except in maturation phase when the cells undergo hypertrophy. Furthermore, the occurrences of chromaffin cells close to the blood vessels indicated a relationship between cells and blood vessels which released their content/hormones into blood circulation.

Very little attention has been paid on the role of adrenocortical tissue in the reproduction of teleosts. Robertson et al. (1961) clearly demonstrated the hyperplasia of the gland at the time of reproduction in many salmonidae. On the other hand Yadav et al.

(1970) reported that in *Heteropheustes fossilis* epinephrine content was higher during reproduction but there was no fluctuation of the non-epinephrine content. According to them, the rise of epinephrine content might be associated with the rise in the level of active phosphorylase required for metabolism during the breeding period.

Reid et al. (1998) opined that in teleosts, chromaffin tissues are associated with the synthesis, storage and secretion of the catecholamines. Sampour (2008) observed that the presence of numerous mitochondria in different shapes in the cytoplasm of chromaffin cells probably produces the energy for activities of the cells during the synthesis of catecholamine hormones. Nussdorfer (1986) suggested that different cytological aspects of interrenal cells could be linked to steroidogenic cells undergoing different degrees of hormonal activity. Civinini et al. (2001) reported that in the male stickleback the interrenal cells had different cytological aspects that could be linked to a steroidogenic cell cycle allowing a periodical renewal of organelles.

In the present investigation, the maximum and minimum gonadal weight was attained in June and October, respectively, coincided with the maximum and minimum diameter of interrenal cells and therefore, the activity of these cells seemed to be closely associated with the gonadal activity. Higher rate of corticosteroid production in fishes at sexual maturity have been reported by Robertson et al. (1961) and Donaldson & Fagerlung (1968). During the post-spawning phase, however, only a few spermatogonial cells, residual spermatozoa were noticed. The gonadosomatic index gradually decreased. Subsequent to release of cytoplasmic contents, the cortical cells become less efficient in gonadal stimulation and were soon transformed into chromophic state. The gonadosomatic index in *N. notopterus* slightly increased during the growth period. The storage of cytoplasmic granules in the interrenal cells began at this moment which was clearly reflected in tinctorial reactions. However, further studies of electron microscopy and quantitative estimation of catecholamine levels will be useful in corroborating the present findings.

Acknowledgements

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